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Award Number: W81XWH-06-1-0575

TITLE: NF-Kappab As a Critical Biological Link Between Psychological Stress and Breast Cancer

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REPORT DATE: November 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-11-2007	Final	1 JUL 2006 - 31 OCT 2007

4. TITLE AND SUBTITLE

Nf-Kappab as a Critical Biological Link Between Psychological Stress and Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0575

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Vanderbilt University
Nashville TN 37232

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

It is a widespread belief that psychological stress is a major factor in breast cancer. However, the biological pathways that link stress to increased breast cancer risk are not well understood. The nuclear factor-kappaB (NF-kB) family of transcription factors is recognized as linking inflammation and immunity to cancer. NF-kB signaling is positioned as a pivotal regulator of aberrant responses that lead to cancer. We tested the hypothesis that NF-kB is a critical biological link between psychological stress and breast cancer. We made innovative use of reporter transgenic mice to measure NF-kB responses to acute and chronic stress and subsequent effects on breast cancer progression. Our data suggest that NF-kB activity is changed in response to both acute and chronic stress and that this impacts both primary tumor formation and subsequent metastasis to the lung.

15. SUBJECT TERMS
NF-kappaB, stress, reporter transgenics

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

It is a widespread, popular belief that psychological stress is a major factor in breast cancer. However, the biological pathways that link stress to increased breast cancer risk are not well understood. As exposure to both acute and chronic stress is a common occurrence, investigation of this link is vital. The nuclear factor-kappaB (NF-κB) family of transcription factors is recognized as linking inflammation and immunity to cancer (1) and multiple studies identify NF-κB activity as important in breast cancer (2). NF-κB signaling occurs in almost every cell type in the body and is positioned as a pivotal regulator of aberrant responses that lead to cancer. Altered NF-κB activity in response to psychological stress in human and mouse models have been reported by several studies (3). We believe that NF-κB activity has multiple functions positioning it as a link between stress and cancer and that this pathway could provide both molecular markers of increased risk and a therapeutic target.

The hypothesis that we proposed to test was that NF-κB is a critical biological link between psychological stress and breast cancer. Our intention was to break new ground in understanding the role of stress in cancer by innovative use of reporter transgenic mice to measure NF-κB responses to acute and chronic stress and subsequent effects on breast cancer progression.

BODY

Specific aim 1: To investigate the effects on NF-κB of acute and chronic psychological stress.

We proposed to use our NGL transgenic reporter mice to determine the pattern of NF-κB activity in response to psychological stress in specific cells in the peripheral blood and in mammary and lung tissue. The models of stress that we proposed to test were to be acute (modeled by restraint) and chronic (modeled by housing). Our proposed methods of analysis were; *in vivo* imaging of live animals, luciferase assay of mammary, lung and peripheral blood extracts, FACS analysis of peripheral blood and measurement of stress hormone levels in plasma.

We have generated NF-κB reporter transgenic mice that expresses a green fluorescent protein (GFP)/luciferase fusion product under the control of a synthetic NF-κB dependent promoter (4,5). As GFP can be detected in individual cells, these mice allow cell specific determination of NF-κB directed transcription. The luciferase moiety of the fusion protein also enables luciferase assay from protein extracts and *in vivo* imaging. These transgenics are named NGL [for NF-κB -GFP/Luciferase] and have the advantage that they enable NF-κB activity to be localized by fluorescence or by immunohistochemical analysis.

We completed a small pilot study to determine if the observations of Bierhaus et al, can be recapitulated using our reporter mice. Mice were restrained for 30 minutes, treated with 1mg/kg norepinephrine via IP injection or pretreated with 1mg/kg prazosin 45 minutes prior to restraint (**Figure 1**). NF-κB activation was monitored 2 hours post treatment using the CCD camera device and photonic counts quantified. Photon counts were quantified specifically in the thorax. This avoids the high basal levels of activity observed in the intestines, skin and brain due to constitutive NF-κB activity in these organs and potential localized effects in the abdominal area due to I.P. injection. The data suggests that NF-κB is activated in mice in response to restraint

stress, that this response can be mimicked by intraperitoneal injection of norepinephrine, and can be inhibited

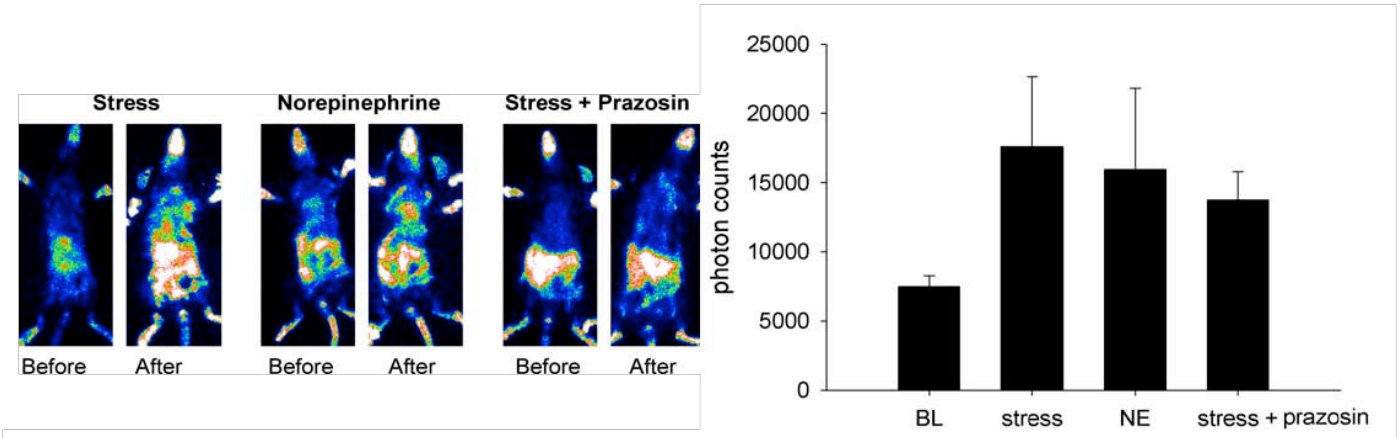

Figure 1. NF-κB dependent luciferase activation in reporter transgenic mice in response to restraint (stress), intraperitoneal injection of norepinephrine (1 mg/kg), or pretreatment with Prazosin 45 minutes prior to restraint. Photon counts were quantified over the thorax (right) in mice at baseline (BL) and following treatment. N=3 mice per group.

by concomitant treatment with Prazosin, an adrenergic antagonist.

Having confirmed that it was likely that NF-□B activity was induced by stress and could be measured using our reporter mice, we decided to further dissect the effects within specific tissues. Experimental NGL reporter transgenics were divided into groups. Control animals were not subjected to treatment. Further groups of mice were placed in a restrainer tube for periods of 10, 30 and 40 minutes. At the time of sacrifice peripheral blood, mammary and lung tissues were harvested, immediately homogenized and processed for luciferase assay (**Figure 2**). Our data shows that NF-□B activity is elevated in mammary tissue in response to restraint stress with the degree of the response being proportional to the length of the acute restraint stress. Results from lung tissue show a similar trend (data not shown). There also appears to be a response in the peripheral blood but in this case, the response is not directly proportional to restraint period. This data indicates that acute restraint stress activates NF-□B activity in peripheral blood and in specific tissues.

We next determined to investigate whether chronic stress also results in elevated NF-□B activity. In some respects, this response is probably more relevant to mammary tumorigenesis than short-term acute stress responses. Housing conditions are well recognized and have been reported in a large number of studies as an effective method of inducing chronic stress in mice. The induction of stress has been reported by either solitary (1/cage) or overcrowded (10/cage) housing conditions as opposed to control social housing (5/cage). Therefore, we assigned transgenic reporter mice into control or stressed housing environment from the time of their weaning and genotyping at 4 weeks of age until harvest at 7 weeks of age. As we had predicted, the chronic stress associated with solitary housing appears to increase NF-□B in both the mammary and lung tissues with a less robust activation being detectable in the peripheral blood (**Figure 3 a-c**). However, to our surprise, our data suggests that the effect of "crowded" housing is not as we had predicted. In this instance the level of NF-□B activity appears to be decreased in the environment with 10 mice to a cage (**Figure 3d**). One potential explanation for the observed result may be that at this relatively young age mice are still very social animals. A mating pair of mice that produces a litter of 8 pups results in a breeding cage that contains 10 animals until the

point of weaning. It is interesting that our current mouse husbandry practice enforces weaning at 3 weeks in order to minimize the stress caused to mice due to overcrowding but that our, admittedly limited, data may suggest that mice may be less stressed if left caged in larger numbers for a longer period. This may be an interesting question for later investigation.

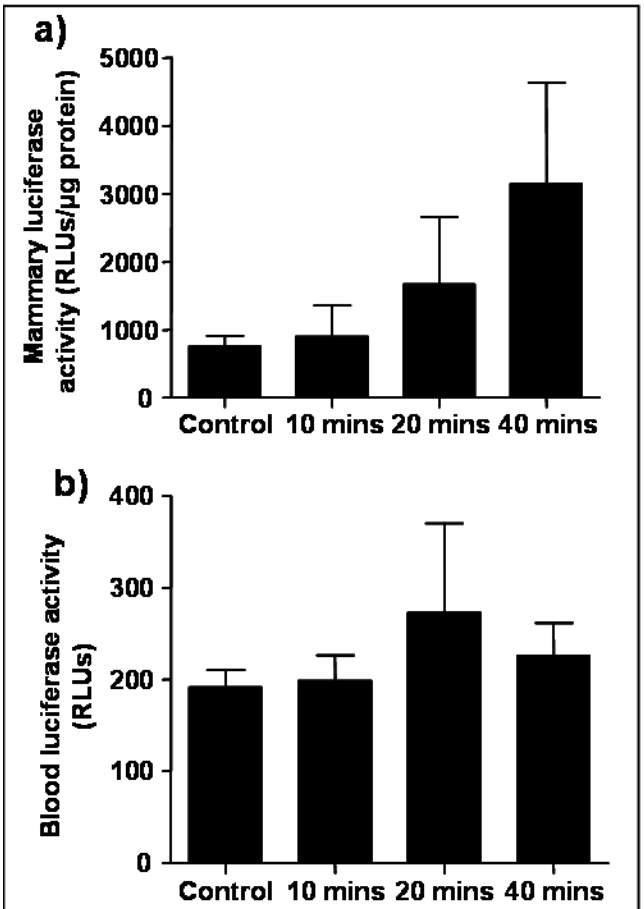

Figure 2. NGL mice were separated into groups and placed in a restraint tube for the indicated periods of time. At the time of sacrifice tissue and peripheral blood were harvested and used for luciferase assay. Results shown are a) mammary luciferase activity expressed as RLUs/µg protein; b) blood cell luciferase activity.

Another component of our original proposal was to investigate the specific cell types in the peripheral blood in which NF-κB is activated in response to stress. In order to complete these studies we induced acute stress in reporter mice by subjecting them to restraint for 30 mins. One hour following the period of restraint we collected peripheral blood from restrained animals and unrestrained controls that was immediately mixed with 0.5M EDTA. We added FACS buffer (1% BSA in PBS) and pelleted cells. The pellets were resuspended in PBS and blocked with Fc block (BD Pharmingen) for 10 mins on ice. In order to identify specific cell types the cells were labeled with the appropriate antibodies; rat anti- mouse CD11b-PE (monocytes/macrophages; BD Pharmingen) or Hamster Anti-mouse CD3epsilon-APC (T cells; BD Pharmingen) and left on ice for 10 mins. Red blood cells were lysed with BD Pharmingen Lyse lysing buffer (BD Pharmingen), and the remaining cells centrifuged and washed with FACS buffer. Samples were resuspended in FACS buffer and analyzed. The results determined that the samples contained approximately 30% monocytes/macs and 50% T cells but we were unable to detect a significant fluorescent signal from GFP. We were uncertain about the potential timing of the NF-κB response in the periphery, therefore we repeated the experiment, restrained the mice for 30 minutes and harvested peripheral blood at 1, 2 and 3 hours post restraint. We were unable to detect significant GFP fluorescence at any time point. Given the relatively low luciferase activity in the peripheral blood we are assuming at this stage that the levels of activation are potentially too low for detection in a small population of cells within the harvested blood. We intend to use a magnetic bead separation strategy to attempt to readdress this issue once we obtain further funding.

We also proposed to measure levels of stress hormones in the serum. We collected peripheral blood by retro-orbital puncture at baseline and at sacrifice and the services of the University Metabolic Core were employed to measure glucocorticoid and norepinephrine levels. No significant differences in levels of either glucocorticoid or norepinephrine were detected as assayed using this methodology (data not shown). We employed a second strategy to measure the stress response in harvested serum. This was a commercially available ELISA assay kit designed to measure norepinephrine (Noradrenaline research EIA, Biosource). Again, no significant differences were detected in response to either housing or restraint stress in the harvested

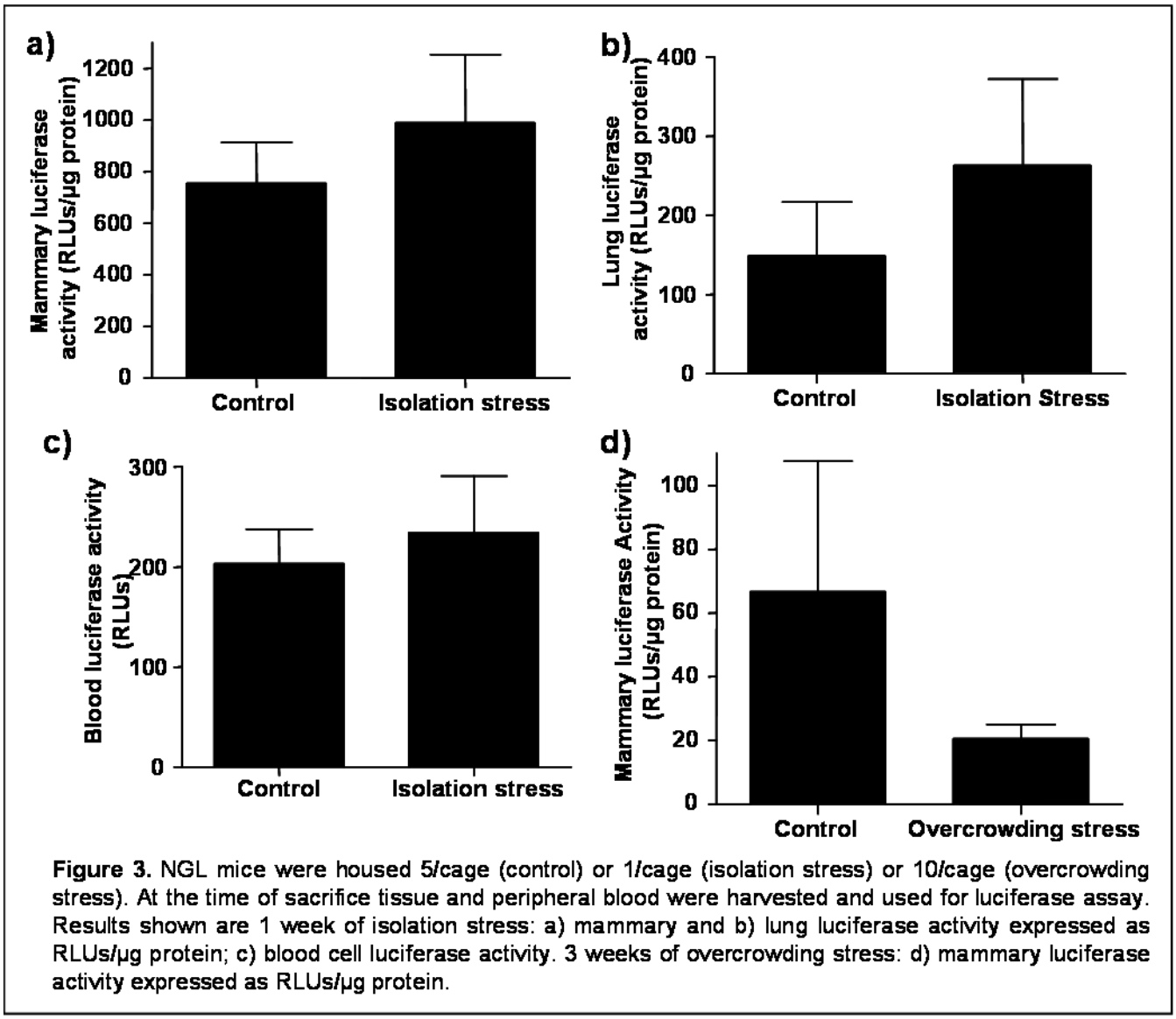

Figure 3. NGL mice were housed 5/cage (control) or 1/cage (isolation stress) or 10/cage (overcrowding stress). At the time of sacrifice tissue and peripheral blood were harvested and used for luciferase assay. Results shown are 1 week of isolation stress: a) mammary and b) lung luciferase activity expressed as RLUs/µg protein; c) blood cell luciferase activity. 3 weeks of overcrowding stress: d) mammary luciferase activity expressed as RLUs/µg protein.

serum (**Figure 4**). It may be that the changes in the levels of stress hormone in the peripheral blood are of relatively small magnitude but still able to elicit significant responses in specific tissues such as mammary and lung. Of note, in a recent publication investigating the effects of stress on ovarian cancer the authors report the use of HPLC tandem mass spectrometry of tissue samples as the methodology to measure hormone changes in response to stress (Thaker at al, Nature Med).

Our accumulated results from this aim suggest that both housing and restraint stress increase NF-□B activity significantly in mammary and lung tissue with a less robust response in the peripheral blood. We have collected a panel of tissue sections from both mammary and lung and intend to use this tissue bank to perform immunohistochemical studies using anti-GFP antibodies to localize the increased NF-□B activity to specific cell types.

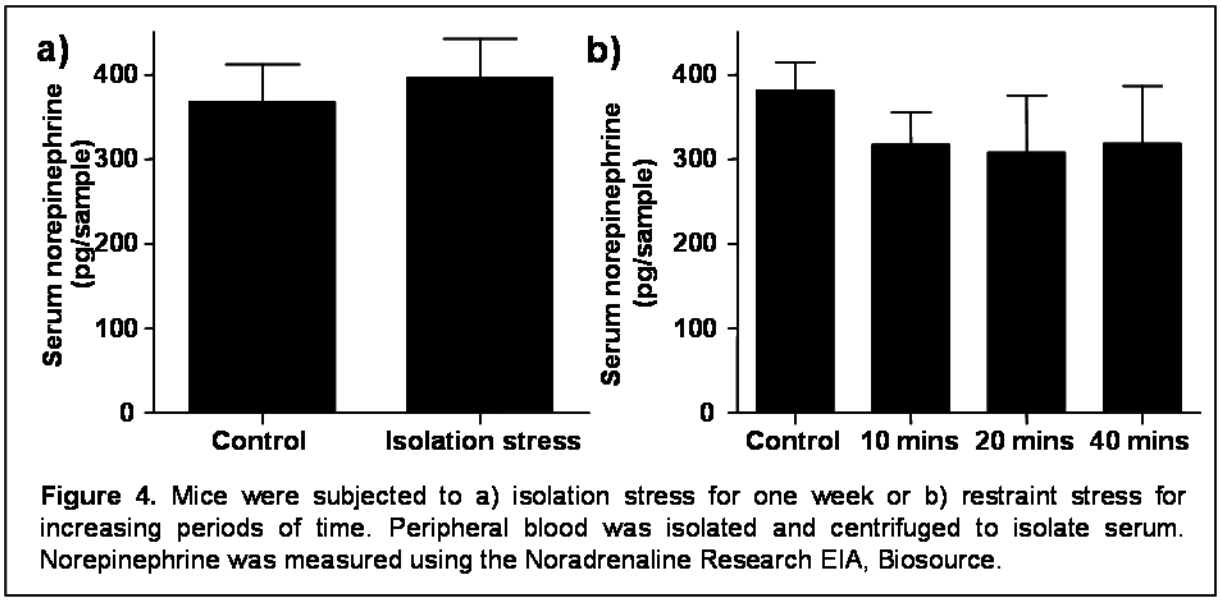

Figure 4. Mice were subjected to a) isolation stress for one week or b) restraint stress for increasing periods of time. Peripheral blood was isolated and centrifuged to isolate serum. Norepinephrine was measured using the Noradrenaline Research EIA, Biosource.

Specific aim 2: To determine if acute or chronic stress exacerbates mammary tumorigenesis and correlates with the NF-□B response.

The data from aim one support our belief that stress results in increased NF-□B activity in tissues relevant both for primary tumor formation and metastasis to the lung. In order to investigate whether this increased activity impacts mammary tumorigenesis we have used an established model of mammary tumor development. Transgenic mice (PyVT) carrying the middle T oncogene under the transcriptional control of the mouse mammary tumor virus promoter/enhancer rapidly (by 7 weeks) produce multifocal mammary adenocarcinomas and secondary metastatic tumors in the lung (by 12 weeks)(5). We obtained the PyVT transgenic mice and established a small colony within our laboratory. While this model of mammary cancer has been used in a number of studies, little is known concerning the pattern of NF-□B activation during the development of the tumors. Our new NGL reporter transgenics provide us with a unique opportunity to investigate the stages during tumor progression in which NF-□B is active and the specific cell types involved. We have crossed the PyVT transgenics with our NGL reporter transgenics to enable us to investigate the pattern of NF-□B activation at the various stages of tumor development; pre-tumor, primary mammary tumor and lung metastasis. For these studies we decided to investigate the effects of housing stress as this significantly alters NF-□B activity and represents chronic stress that could be argued to be the most relevant with respect to impact on the human population. Mice were weaned and genotyped at 3 weeks old. At 4 weeks old animals were assigned into the different housing environments (1, 5 or 10 mice per cage). At 7 weeks old mammary glands were harvested in order to investigate the effects of stress on NF-□B signaling in the environment of tumor development. Western analysis of nuclear extracts show that there are higher levels of p65 in the nucleus in mice in solitary housing (**Figure 5a**). This is a classically accepted measure of increased NF-□B signaling. We also performed luciferase assays on mammary tissue extracts from mice in control housing (5/cage) and in stressed housing (1 and 10/cage)(**Figure 5b**). As we had originally predicted the levels of NF-□B activity were relatively higher in the stressed solitary housed animals. Again unexpectedly, but in parallel with our results from Aim 1, the levels of NF-□B activation were actually lower in the mice that were kept in overcrowded housing. Luciferase assays were also performed on mammary tissue extracts harvested at 12 weeks old. By this time point the levels of NF-□B activity in all housing conditions were similar and uniformly high (data not shown). As levels of NF-□B activity are known to be high in both human mammary tumors and in mammary

tumor cell lines, we suspect that this reflects the high tumor burden by this stage which obscures the smaller effects induced by stress.

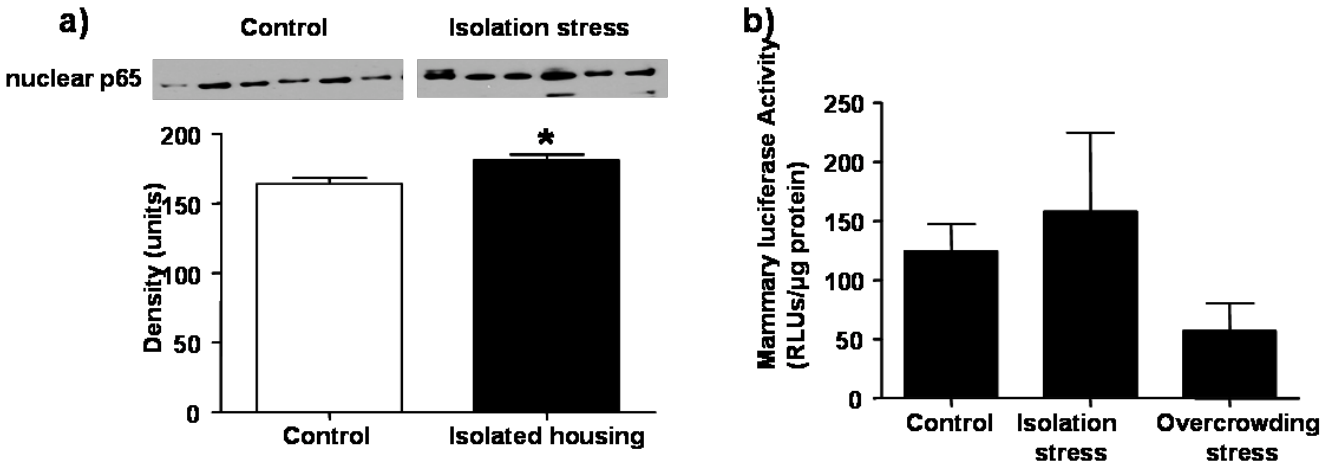

Figure 5. PyVT mice were subjected to control housing (5/cage), isolated housing (1/cage) or overcrowded housing (10/cage) from 4 to 7 weeks old and mammary glands harvested. a) Nuclear extracts were prepared from mammary glands of control and isolation stress groups. Western blot was performed for p65. Bands were quantified by densitometry. Data are represented as mean ± S.E. density. *indicates $p < 0.05$, significantly different from control; $n = 6$. b) Mammary glands were homogenised and luciferase assay performed. Luciferase activity is expressed as RLUs/µg protein.

At 12 weeks old, all mammary glands were harvested and the tumor load was assessed. Our original intent was to quantify tumor numbers by preparing whole mount preparations and counting tumor numbers. We completed these studies but discovered that there was a much greater degree of variability in the PyVT model than we had originally anticipated. Within the same conditions some mice would have a single large tumor whereas others would have multiple much smaller tumors. This resulted in an extreme level of variability within experimental sets and would necessitate much larger experimental groups in order to approach significant differences. Therefore, we assessed tumor burden as total mammary weight (**Figure 6**). Our original hypothesis would have predicted a greater tumor load in stressed mice correlated with increased levels of NF-□B activity. What we observed was the opposite effect, in that solitary housed mice in which we had shown NF-□B activity to be elevated had a lower tumor burden. In addition, the overcrowded housing, in which the NF-□B activity appeared to be lower than detected in controls is tending towards a greater tumor load.

At the same 12 week time point lungs were harvested and inflated to count surface macro-metastases. Lung tissue was fixed in Bouin's fixative for 24 hours and 70% ethanol prior to embedding for future sectioning to examine micrometastases and for immunohistochemical studies. We normalized the number of lung metastases to the total mammary gland weight as representative of primary mammary tumor burden. The data suggests that, in agreement with our original hypothesis, there is a strong trend towards great numbers of lung metastases in mice that were solitary housed and in which NF-□B activity was elevated (**Figure 7**). In addition, the mice from overcrowded housing in which our data suggests that NF-□B activity is lower than in controls also have decreased numbers of lung metastases. Therefore, both sets of housing data in this case

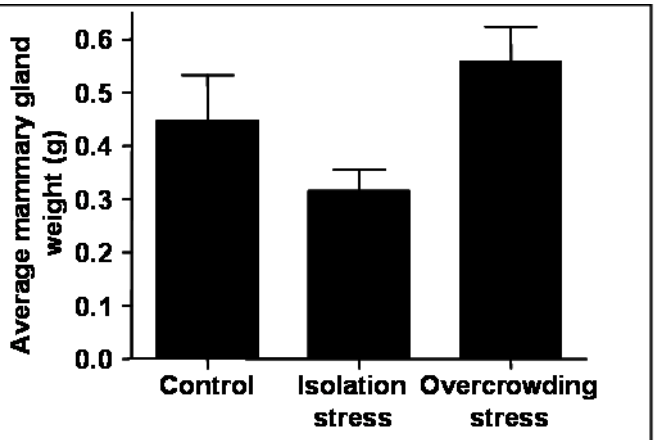

Figure 6. PyVT mice were subjected to 5/cage (control), 1/cage (isolation) or 10/cage (overcrowding) housing conditions from 4 to 12 weeks old. Mammary glands were collected and weighed and the average mammary gland weight calculated for each condition.

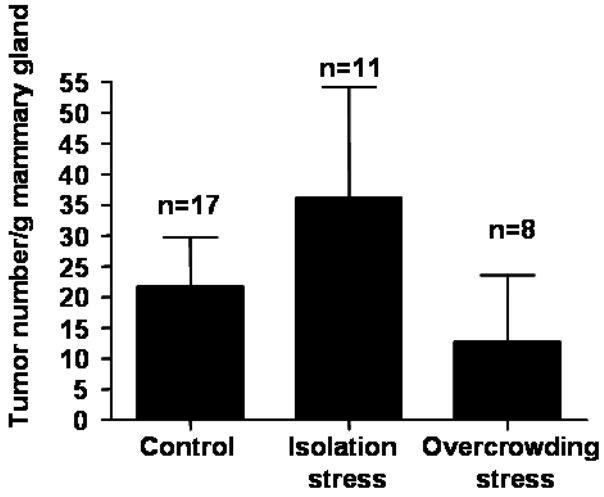

Figure 7. PyVT mice were subjected to 5/cage (control), 1/cage (isolation) or 10/cage (overcrowding) housing conditions from 4 to 12 weeks old. Lungs were harvested, inflated in Bouins solution and surface tumor number counted. Tumor number was normalised/g of mammary tissue.

agree with our original hypothesis that numbers of lung metastases would correlate with levels of NF-□B activation.

As we discovered that the PyVT model exhibits more variability than we had originally anticipated we have established a novel mouse mammary tumor cell line from PyVT mice crossed with our NGL NF-□B reporter transgenics. These mammary tumor cells carry an inbuilt GFP/luciferase reporter of NF-□B activity and are termed PYG. We have generated two separate lines that exhibit different growth characteristics (**Figure 8a and b**). We have tested their response to known activators of NF-□B activity such as TNF-alpha to confirm that they are still functional reporters of NF-□B activation (**Figure 8c**). As these cells have constitutive NF-□B activity they can be tracked by imaging for luciferase activity (light production) using IVIS (Zenogen) in intact mice after treatment with luciferin. *In vivo* growth of these tumor cells in the lung can be monitored following injection of 500,000 cells into the tail vein (**Figure 8d**). Both tumor cell lines are capable of producing visible lung metastases 5 weeks post tail vein injection (eg. 8.75 ± 2.462; n=4). We intend to utilize these cells in future studies to assess *in vitro* responses to treatment with stress hormones such as norepinephrine. Injection of a defined number of these cells into the mammary fat pad will enable more controlled assessment of the effects of stress on primary mammary tumor development. In addition, we intend to complete further studies in which these cell lines will be injected via the tail vein into experimental animals under different housing conditions to assess effects on the metastatic phase of tumor development while having a more defined input tumor cell population.

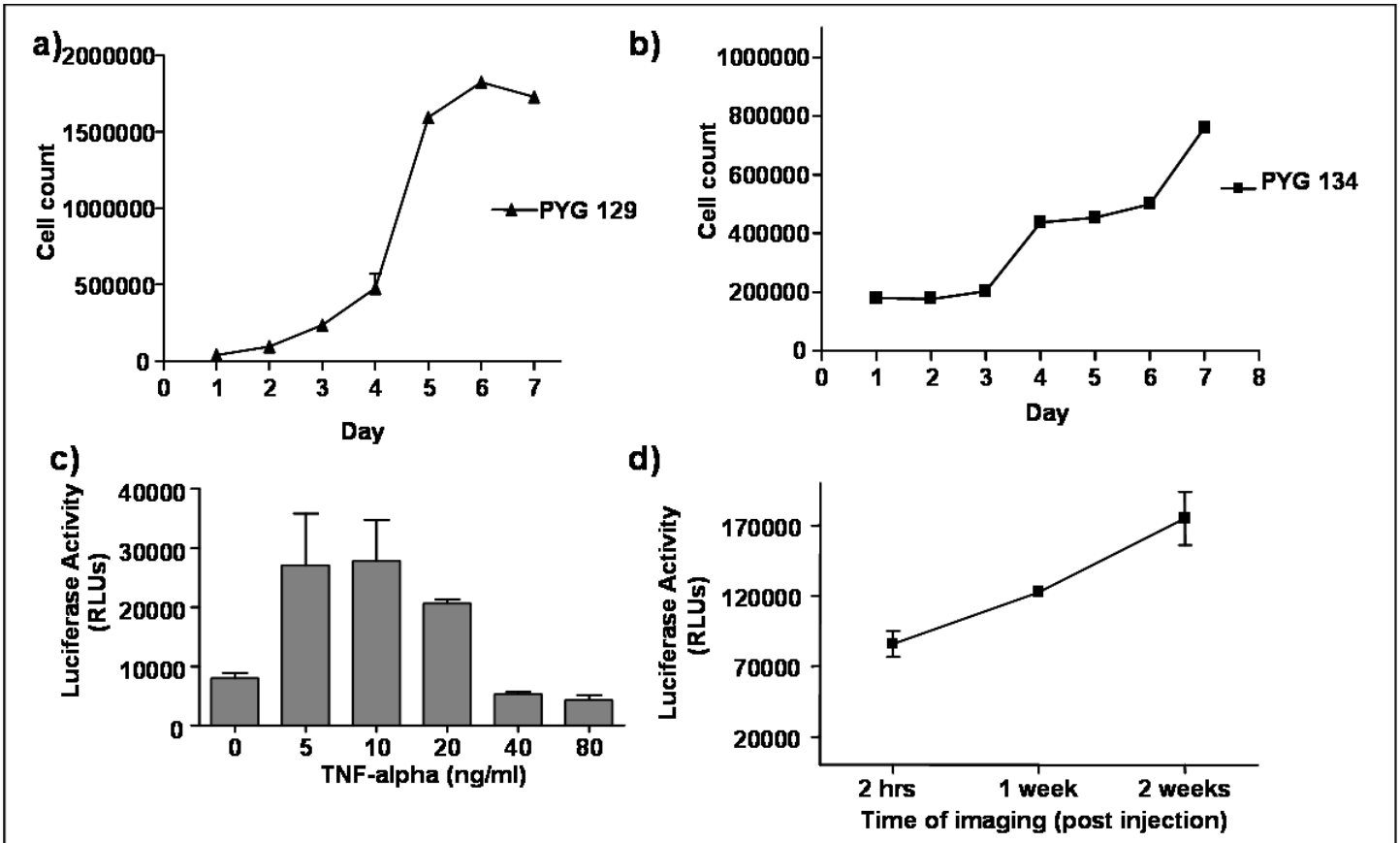

Figure 8. PYG cell growth curves for line 129 (a) and 134 (b). (c) Luciferase activity of PYG 129 cells 4 hrs after addition of increasing concentrations of TNF-α. (d) PYG 129 cells were injected via the tail vein. Mice were injected with luciferin and imaged using IVIS (Zenogen).

The cumulative data from this section suggests that NF-□B activity is increased during early stages of mammary tumor development in response to solitary housing stress. Unexpectedly, overcrowded housing appears to result in decreased NF-□B activation in early stages of tumor development. The NF-□B response to housing stress is obscured at later stages of tumor development, probably due to the high primary tumor load. As predicted the levels of NF-□B activity appear to directly correlate with lung tumor metastasis numbers. However, in opposition to our original hypothesis, the level of NF-□B activity may be inversely proportional to primary mammary tumor load at early stages of development. One possible explanation for this observation may be that at these early stages, activation of NF-□B contributes to more effective immunosurveillance but at this stage we have no direct evidence in support of this idea.

KEY RESEARCH ACCOMPLISHMENTS

1) We have validated the use of our NGL reporter transgenics as an effective model to monitor the effects of both acute and chronic stress on NF-□B activity.
2) We have obtained data that suggests that stress has effects on levels of NF-□B activity and that these then impact mammary tumorigenesis both at the stage of primary tumor development and during metastasis to the lungs.
3) We have established two novel mammary tumor cell lines that carry an in built reporter of NF-□B activity and are metastatic to the lungs. These cell lines will be a valuable resource for future studies.

4) We have obtained important preliminary data for future grant applications. We have applied for future funding to continue this area of research from the Susan G Komen Foundation and the Veterans Administration and intend to apply for further DOD funding support next year should neither of the other two attempts at securing funding be successful.

REPORTABLE OUTCOMES

Connelly, L., Saint-Jean, L., Sherrill, T., Fingleton, B., Newsome, A., Pigg, R., Yull, F. Linking NF-kappaB activation with psychological stress: effects on tumorigenesis. 7th annual host-tumor interactions program and Department of Cancer Biology Joint Retreat, Nov 16-17, Lake Barkley, Kentucky. Poster presentation awarded first place prize.

We have started to combine the data in the form of a publication and are optimistic that our results will be published in the near future.

We have established two novel mammary tumor cell lines that carry an in built reporter of NF-□B activity and will be very valuable for future studies.

CONCLUSIONS

In summary, we have used our unique tools to provide pilot data suggesting that psychological stress impacts breast cancer via alteration of NF-□B signaling. We have determined that acute restraint stress and solitary housing stress increases level of NF-□B activity. Unexpectedly, we find that overcrowded housing in relatively young mice appears to result in decreased levels of NF-□B activation. The evidence from these pilot studies suggests that chronic stress induced by solitary housing is likely to be the best methodology for future studies of the effects of stress on NF-□B activity and mammary tumorigenesis. Our studies using the PyVT model suggest that increased NF-□B activity in response to stress may result in a decrease in primary tumor formation but an increase in metastasis. The reverse correlation is observed in housing situations that result in lower NF-□B activation. This opens the intriguing possibility of differential effects of NF-□B signaling during specific stages of tumor development.

Recent evidence suggests that chronic behavioral stress results in higher levels of tissue catecholamines, greater tumor burden and a more invasive phenotype, recently reviewed by Thaker et al, 2007 (7). A study into the relationships between acute social stress, immunological alterations and the development of pulmonary metastases of B16F10 melanoma has demonstrated the ability of social stress to result in increased numbers of pulmonary metastases and altered the serum level of corticosterone (8). In addition, a recent extensive study reported that chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian cancer (9). This orthotopic model introducing ovarian cancer cells into mice subjected to either restraint or housing stress demonstrated that both models of stress resulted in increased tumor number. The levels of catecholamines were elevated in mouse tissues and similar effects could be induced by isoproterenol (a non-specific agonist B-adrenergic agonist) and blocked by propranol (an antagonist of adrenergic signaling). The authors also investigated the downstream mechanisms contributing to the observed effects and identified effects on VEGF and angiogenesis as contributing to the increased tumor number. Interestingly, this study did not investigate effects on NF-□B activity despite the fact that VEGF is known to be a downstream target of this signaling pathway. The authors also completed a comprehensive investigation of direct effects of adrenergic signaling on the ovarian cancer cells *in vitro*, coming to the conclusion that the effects were mediated at least in part by

direct stimulation of adrenergic signaling pathways within the ovarian cancer cells. We believe that the "missing" link between stimulation of adrenergic signaling pathways by stress and induction of downstream targets such as VEGF is NF-□B activity. Clearly further investigation is necessary to dissect the importance of these signaling pathways in particular cell types that are activated in response to stress and their potential long term pathological effects.

The preliminary data obtained during the course of these studies provides critical proof-of-principle information linking chronic stress to activation of the NF-□B pathway and suggesting an impact on mammary tumorigenesis. While maintenance of normal immune surveillance may be important for protection against tumors, persistent inflammation is likely to be counterproductive. Information gained in future studies regarding specific pathways and mediators that influence tumor risk in mouse models could be applied to human chemoprevention studies, particularly in high-risk populations. Our models could be used to test the possibility that drugs currently in use for treatment of anxiety/stress may have efficacy in cancer prevention.

We intend to use the pilot data obtained with the support of this grant funding to apply for support for future studies to: 1) provide evidence that psychological stress has differential effects on NF-□B signaling in specific cell types, 2) demonstrate the potential for NF-□B signaling in specific cell types to contribute to tumor development, 3) identify NF-□B as a novel biomarker that could be used as an indication of increased cancer risk and the need for intervention, 4) determine whether modulation of NF-□B activity in specific cell types could be therapeutic.

REFERENCES

1. Karin M, Greten FR. (2005) NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005 5:749-59.
2. Wu JT ,Kral JG . (2005) The NF-kappaB/IkappaB signaling system: a molecular target in breast cancer therapy. J Surg Res 123:158-69.
3. Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, Ferstl R, von Eynatten M, Wendt T, Rudofsky G, Joswig M, Morcos M, Schwaninger M, McEwen B, Kirschbaum C, Nawroth PP. (2003) A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci U S A. 100:1920-5.
4. Yull, FE, Han, W, Jansen, ED, Everhart, MB, Sadikot, RT, Christman, JW and Blackwell, TS. 2003. Bioluminescent detection of endotoxin effects on HIV-1 LTR-driven transcription In Vivo. Journal of Histochemistry and Cytochemistry 51:1-9.
5. Everhart MB, Han W, Sherrill T, Arutinov M, Polosukhin VV, Burke JR, Sadikot RT, Christman JW, Yull FE, and Blackwell TS. (2006) Duration and intensity of NF-kB activation determine the severity of endotoxin-induced acute lung injury. J Immunol 176:4995-5005.
6. Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, Pollard JW. (2003) Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol. 163:2113-26.
7. Thaker PH, Lutgendorf SK, Sood AK. (2007) The neuroendocrine impact of chronic stress on cancer. Cell Cycle 6:430-3.
8. Vegas O, Fano E, Brain PF, Alonso A, Azpiroz A. (2006) Social stress, coping strategies and tumor development in male mice: behavioral, neuroendocrine and immunological implications. Psychoneuroendocrinology. 31:69-79.

9. Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R, Lu C, Jennings NB, Armaiz-Pena G, Bankson JA, Ravoori M, Merritt WM, Lin YG, Mangala LS, Kim TJ, Coleman RL, Landen CN, Li Y, Felix E, Sanguino AM, Newman RA, Lloyd M, Gershenson DM, Kundra V, Lopez-Berestein G, Lutgendorf SK, Cole SW, Sood AK. (2006) Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med. 12:939-44.

APPENDICES

Personnel receiving salary support
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